

# Persistence and Mobility of Tebufenozide in Forest Litter and Soil Ecosystems under Field and Laboratory Conditions

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**Abstract:** A field microcosm study was conducted to determine persistence of tebufenozide, an insect growth regulator, in sandy litter and soil. Litter and soil plots (c. 4.5 m<sup>2</sup> each) were sprayed with an aqueous suspension concentrate formulation of tebufenozide at rates of 35, 70 and 140 g AI ha<sup>-1</sup>. Samples were collected at intervals up to 408 days after spraying, and analyzed for tebufenozide residues. The data were subjected to regression analysis and half-life (DT<sub>50</sub>, the time required for 50% of the initial residues to disappear) values were computed. The DT<sub>50</sub> was c. 62 days for both substrates treated with the two lower dosage rates. At the highest dosage rate, the DT<sub>50</sub> was 115 days for the litter and c. 52 days for the soil, indicating irregular variations in persistence. Downward movement in soil occurred only in trace amounts, suggesting strong adsorption.

Laboratory microcosm studies were conducted to investigate the relative importance of rainfall, exposure to light and volatilization on persistence. Vertical movement occurred in litter and soil (both sandy and clay types) during rainfall. The amount moved increased with the amount of rainfall, but decreased with the rain-free period. The larger the rain droplets, the greater the downward movement. When the rainwater could move laterally along the surface of the substrate (as would occur on a slope), more lateral movement than vertical movement of tebufenozide occurred. The photolysis study indicated that disappearance of tebufenozide was directly related to the duration of exposure to radiation and radiation intensity. Volatilization of tebufenozide depended upon the ambient temperature and the duration of air passing through the substrates. Nonetheless, the amount lost by volatilization was much lower than the amount lost after rainfall or exposure to radiation, thus indicating the greater influence of rainfall and sunlight on persistence.

In the laboratory microcosm studies, more tebufenozide was lost from the sandy substrates than from the clay substrates. This behaviour was attributed to the greater adsorptive capacity of the clay substrates, thus providing a greater protection against downward mobility and loss due to radiation.

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## 1 INTRODUCTION

Sustainable development of forestry resources is vital to Canada's economic growth. Protection of these resources requires the use of insecticides to reduce losses caused by insect pests. Efforts are being undertaken to discover and implement selective insect-control methods that are environmentally acceptable, instead of relying on conventional broad-spectrum insecticides. Insect

growth regulators have some selectivity, and these compounds include juvenile hormones, anti-juvenile hormone analogs, moulting hormone (ecdysone) agonists and chitin synthesis inhibitors.

The hydrazine derivative, tebufenozide ('Mimic'; RH-5992; *N*-*tert*-butyl-*N'*-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide) is a novel type of insect growth regulator interfering with the moulting process of lepidopteran insects. When ingested, it acts as an agonist

or mimic of the insect moulting hormone, 20-hydroxyecdysone, by causing cessation of feeding, premature ecdysis and eventual death. The material was found to be lepidopteran-specific in laboratory studies.<sup>1,2</sup> This property makes tebufenozide a desirable insecticide for forestry use to manage lepidopteran insect populations. In laboratory studies, tebufenozide has shown appreciable toxicity to the eastern spruce budworm,<sup>3</sup> *Choristoneura fumiferana* (Clem.), a defoliator that causes considerable damage to the spruce–fir forests in eastern North America. Recent aerial field trials have also shown that the material is effective in controlling the spruce budworm (Cadogan, pers. commun.).

Before an insecticide can be used operationally in Canada, it must be registered under the Pest Control Products Act (PCPA), and this requires that the chemical should not have any significant adverse impact on environmental quality or on human health. Literature information is sparse on the environmental chemistry and behaviour of tebufenozide in forest litter and soil under Canadian conditions. Recently, a field study was conducted on persistence and mobility of tebufenozide in clay loam soil and the litter overlay<sup>4</sup> (described in this paper as the ‘clay soil’ and ‘clay litter’ respectively). The present paper reports a field microcosm study involving sandy loam soil and the corresponding litter overlay (referred to as the ‘sandy soil’ and ‘sandy litter’ respectively). The purpose of this study was to understand (a) the persistence of tebufenozide in forest litter and soil of low clay content following application using simulated aerial sprays, and (b) vertical mobility in the soil.

In addition to the field study, several laboratory microcosm studies were undertaken on the same sandy litter and soil, and also on the clay litter and soil reported previously.<sup>4</sup> The purpose was to examine the effects of (i) rainfall on vertical and lateral mobility of tebufenozide in litter and soil samples of two different types, (ii) light radiation on photostability and (iii) volatilization from the two substrates on disappearance. The objective was to determine the relative importance of these three factors under controlled conditions in the laboratory, and to compare with the field behaviour of tebufenozide.

## 2 MATERIALS AND METHODS

### 2.1 Study I—Behaviour of tebufenozide in sandy litter and soil ecosystems under field conditions

#### 2.1.1 Site description

The study was conducted about 5 km east of Greenwater Lake, which is located c. 80 km northwest (46°53' N,

84°03' W) of Sault Ste. Marie, ON, Canada. The area contained white spruce [*Picea glauca* (Moench) Voss] trees of varying heights (2–8 m). The forest floor was flat and covered with grass and moss patches. In the open areas of the treatment block (c. 0.8 ha), four litter and four soil plots (c. 4.5 m<sup>2</sup> each) were selected. Three plots were used for tebufenozide treatment, and the fourth served as the control. Small objects such as fallen branches, twigs and small stones were removed from the plots, and the surface was levelled and packed to the original condition. The overlying litter, moss and organic detritus were also removed to a depth of c. 10 cm in the soil plots to fully expose the underlying mineral soil to spray deposits.

#### 2.1.2 Spray application

Plots were treated at rates of 35, 70 and 140 g tebufenozide ha<sup>-1</sup> (operational rates are 70–140 g ha<sup>-1</sup>). Appropriate amounts of a tebufenozide 240 g litre<sup>-1</sup> SC (RH-5992 2F; Rohm and Haas Co.) were mixed with distilled water containing 2 g litre<sup>-1</sup> Rhodamine WT dye (technical grade, A. S. Patterson, 1110 Shepherd Ave. East, Suite 404, North York, ON, Canada), to produce the spray-mixes. A portable shelter (polyethylene sheets fixed onto wooden frames) enclosing an area of 2.2 × 2.2 m to a height of c. 0.5 m was placed around each plot before spray application, and was removed about 20 min after spray. The shelter helped to prevent drift and hence contamination of the neighbouring plots. The volume rate used was 2 litre ha<sup>-1</sup> for all three dosage rates, and application was made on 2 June 1992 between 0630 and 1100 h, using a hand-held, battery-powered ‘Flak’<sup>®</sup> rotary atomizer (Micron Agri-Sprayers Canada, Walkerton, ON). During spraying, the average temperature, relative humidity, wind speed and cloud cover were 11.8°C, 85%, 5.2 km h<sup>-1</sup> and 1/10 respectively, and there was no precipitation.

#### 2.1.3 Droplet size spectra and deposit assessment

For droplet measurements, two Kromekote<sup>®</sup> cards (K-card; Intercity Papers Ltd, Mississauga, ON, Canada) (each 5.0 × 7.5 cm) were mounted side by side on an aluminium (Al) sheet (12 × 12 cm). For assessment of spray mass deposit, two glass plates (each having the same dimensions as the K-card) were mounted on another Al sheet. The two Al sheets were joined together by a heavy duty masking tape. Four of these units (described as the K-card/glass plate units) were placed on the ground of each treatment plot, one to each corner. The samplers were laid out c. 15 min prior to spray application, and were collected at 30 min post-spray. The K-cards were wrapped in Al foil and stored in a desiccator. The glass plates were eluted with acetonitrile and the eluates were collected in amber-coloured glass bottles, stored initially on dry ice, and later at -20°C in the freezer.

#### 2.1.4 Collection of sandy litter and soil samples

Sandy litter and soil samples were collected from the three treatment plots at 1 h after spray (described as the zero time) and at 4, 9, 16, 23, 31, 43, 52, 64, 85, 107, 135, 169 (prior to snowfall), 357, 370 and 408 days post-spray (after snowmelt in the spring of 1993). Litter samples were taken as single cores per sampling interval by driving a metal frame,  $15.5 \times 15.5 \times 5.0$  cm, into the ground and removing the contents with a clean trowel. Three samples were taken at each time interval. Each sample was packed separately in Al foil, stored at  $-20^{\circ}\text{C}$  and brought to the residue laboratory. In the laboratory, each litter sample was macerated by passing through a Hobart® chopper after removing the stones, twigs, roots, etc., passed through a 2-mm sieve, mixed well and stored at  $-20^{\circ}\text{C}$  until analysis.

Soil samples (sandy loam, depth 0–15 cm) were collected by using a heavy duty stainless steel auger developed in our laboratory. The auger was constructed of a stainless steel tube (diameter 5.0 cm, length 50 cm) split open lengthwise, and had a sharpened bevel at one end as a cutting edge. The two halves were held together by tongue and groove channels along their length and locked in place with screw clamps. A solid stainless steel cap was placed over the head of the auger and a sliding cylindrical stainless steel rod (length 50 cm, diameter 2.5 cm) was passed through a handle. The auger was driven into the soil to a depth of 15 cm with a sledge hammer. The auger was then drawn out by twisting and pulling the handle, without disturbing the surrounding soil, and the tube was split open. The core was sliced with a knife (cleaned between slices) into four segments corresponding to 0–2.5 cm, 2.5–5.0 cm, 5.0–10.0 cm and 10.0–15.0 cm. Three soil cores were taken from each of the three dosage (35, 70 and  $140 \text{ g AI ha}^{-1}$ ) plots at each sampling period. The auger was cleaned between sampling to avoid contamination. The soil core segments belonging to the same dosage level and depth were pooled to form a composite sample for residue analysis. The samples were sieved (2-mm openings) to remove stones, twigs, roots, etc., wrapped in Al foil, placed in containers with dry ice, and brought to the residue laboratory where they were stored at  $-20^{\circ}\text{C}$  until analysis.

#### 2.1.5 Extraction and analysis

Analytical grade tebufenozide (purity  $> 99.6\%$ ) was provided by Rohm and Haas Company. The samples were processed as described by Sundaram,<sup>5</sup> and analyzed for residues using a high-performance liquid chromatographic (HPLC) method.<sup>6</sup> Briefly, aliquots of the samples were extracted with a mixture of acetone and water; the crude extracts were solvent-partitioned, concentrated and cleaned by using Florisil® or Prepsep®-NH<sub>2</sub> columns. After fractional elution of the columns with hexane + ethyl acetate as the eluent, the eluates were analyzed by HPLC using a diode-array

UV detector set at 236 nm, and an RP-8, 10- $\mu\text{m}$  column with a mobile phase of acetonitrile + dioxane + water. Mean recoveries after fortifying the pre-spray and control samples with tebufenozide at 0.05 to  $1.0 \mu\text{g g}^{-1}$  ranged from 94 to 102%, with coefficients of variation from 5 to 10%. All measurements were corrected for recovery efficiency. The limit of detection (LOD) and limit of quantification (LOQ) for both substrates were 0.020 and  $0.050 \mu\text{g g}^{-1}$  respectively. None of the pre-spray or control samples contained any tebufenozide, and there was no interference from co-extracted materials. To investigate the storage stability of tebufenozide, processed pre-spray and control sample extracts were fortified with varying levels of the analyte and stored at  $-20^{\circ}\text{C}$  for 60 days. During this period, aliquots were taken and analyzed.<sup>6</sup> The recoveries were quantitative for a 30-day period, beyond which a gradual decrease in recovery with time was noted, ranging approximately from 5% on the 42nd day to 8% on the 60th day. Radiotracer studies using [<sup>14</sup>C]tebufenozide showed negligible adsorption onto polyethylene, glass or Teflon® surfaces. Moisture content of the substrates was determined<sup>7</sup> by taking 5.0-g aliquots of the processed samples ( $n = 3$ ) and drying them in a thermostatic oven at  $120^{\circ}\text{C}$ .

#### 2.1.6 Analysis of droplet stains on Kromekote cards (K-cards)

The droplet stains on the K-cards were analyzed as described by Sundaram *et al.*<sup>8,9</sup> using an American Optical Microscope at magnifications of  $25\times$ ,  $40\times$  and  $100\times$ . The minimum detection limit (MDL) was  $25 \mu\text{m}$  for the stains on the K-cards (corresponding to  $11 \mu\text{m}$  in diameter for the spherical droplets). The data from 24 cards [(four cards  $\times$  three litter plots) + (four cards  $\times$  three soil plots)] were grouped according to size category, to calculate the maximum and minimum diameters ( $D_{\text{max}}$  and  $D_{\text{min}}$  respectively), number and volume median diameters ( $D_{\text{N},5}$  and  $D_{\text{V},5}$  respectively) and droplet density (droplets  $\text{cm}^{-2}$ ).

#### 2.1.7 Deposit recovery on glass plates

The deposits obtained on glass plates were extracted with acetonitrile, and the extracts were concentrated by flash-evaporation prior to analysis of tebufenozide.<sup>6</sup> The data obtained for the litter and soil plots were averaged and converted into  $\text{g AI ha}^{-1}$ .

### 2.2 Study II—Behaviour of tebufenozide in sandy/clay litter and soil samples in laboratory microcosms

Samples of sandy litter and soil were collected from the same location as in Study I, processed and packed in Al pans (household turkey-roasting pans,

38 × 27 cm × 7 cm high), to a depth of 4 cm. The pans were covered with plastic sheets to minimize evaporation of moisture from the samples, and stored in a cold room at  $-10^{\circ}\text{C}$  until ready for use. Samples of clay litter and soil were collected from a location<sup>4</sup> in Laird Township Municipality ( $46^{\circ}23' \text{ N}$ ,  $84^{\circ}01' \text{ W}$ ; about 30 km southeast of Sault Ste. Marie, ON, Canada). The samples were processed, packed in Al pans and stored as described above.

Aliquots of the formulation, RH-5992 2F, were mixed with water to provide a concentration of  $5 \text{ mg AI ml}^{-1}$ . Calculated amounts of the resulting suspension were mixed thoroughly with litter samples (4 cm deep) in one of the Al pans to provide an initial concentration of  $10 \text{ mg AI kg}^{-1}$  wet weight. Two Al pans containing untreated litter (4 cm deep) were taken out of the cold room and allowed to warm up to room temperature ( $20 (\pm 1.5)^{\circ}\text{C}$ ). Over the 4-cm untreated litter sample, the tebufenozide-treated litter was packed to a height of 2 cm. Replicate pans were prepared just before use to minimize storage problems. The Al pans containing untreated forest soil samples (4 cm deep) were also packed in a similar manner with a 2-cm layer of treated soil containing tebufenozide at  $10 \text{ mg kg}^{-1}$  wet weight.

### 2.2.1 Effect of cumulative rainfall on vertical mobility of tebufenozide in litter and soil

Prior to the investigation, test trials were conducted to determine persistence of tebufenozide in the top 2-cm layer of litter and soil (both clay and sandy types) in the Al pans kept in darkness at  $15^{\circ}\text{C}$  in an environmental chamber without any rainfall. No loss occurred even up to 12 days after treatment, with little vertical mobility into the bottom untreated layers.

To determine vertical mobility of tebufenozide due to rain, simulated rainfall was generated in a spray

chamber ( $4.3 \times 0.9 \times 3.05 \text{ m}$ ) using a Veejet® 8002 nozzle having an orifice of 0.90 mm and a spray angle of  $74^{\circ}$  (Spraying Systems Co., Wheaton, Illinois, USA). The rainfall application parameters (spray pressure 180 kPa, flow rate of rain water  $0.58 \text{ litre min}^{-1}$ , rainfall intensity  $5 \text{ mm h}^{-1}$ ) were chosen by repeated trials to provide 20 and 40 mm amounts of rain over the Al pans containing the litter and soil samples (Fig. 1), at a rain-free period of 2 days after tebufenozide treatment. Two types of application were used: (i) continuous rainfall for 4 and 8 h to provide 20 and 40 mm cumulative rain respectively and (ii) intermittent rainfall at four times (with an interval of 3 h in between), each time for 1 and 2 h to provide 20 and 40 mm rain respectively. A rain gauge was used to monitor the amount of rainfall, and rain droplet size spectra (diameter range 80 to  $1100 \mu\text{m}$ ,  $D_{N,5}$   $445 (\pm 85) \mu\text{m}$ ,  $D_{V,5}$   $780 (\pm 95) \mu\text{m}$ ) were measured by collecting the droplets in castor oil.<sup>10</sup> Immediately after the rain, the Al pans were removed from the spray chamber. Samples (cores, 6 cm deep and 1 cm in diameter) were taken from the pans and sliced into top 2-cm, middle 2-cm and bottom 2-cm layers. The cores from corresponding layers were pooled for analysis of tebufenozide. Some of the samples were found to be soaked with the rain water, and they were filtered under suction using PTFE™ membrane filters [ $1\text{-}\mu\text{m}$  pore, Product No. TF-1000 (66159), Gelman Sciences, Ann Arbor, MI 48106, USA] to remove the excess water. The filtered water and the moist litter/soil samples were both analyzed for tebufenozide residues.<sup>6</sup>

### 2.2.2 Effect of rain-free period on vertical mobility of tebufenozide in litter and soil

The Al pans containing the litter and soil substrates as above were subjected to 30 mm rainfall at two rain-free

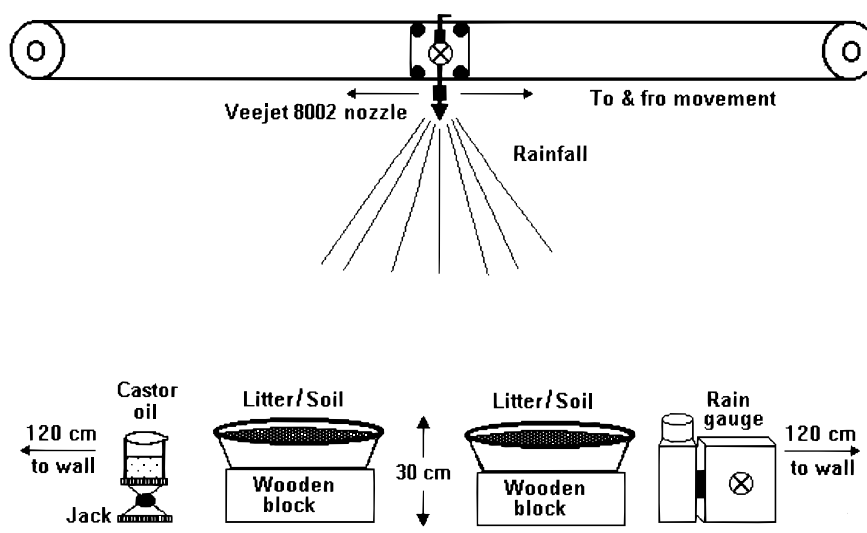


Fig. 1. Arrangement of litter/soil substrates in the spray chamber for exposure to simulated rainfall.

periods of 3 and 12 days after treatment. After the rain, samples were processed and analyzed for tebufenozide residues.<sup>6</sup>

### 2.2.3 Effect of rainfall intensity on vertical mobility of tebufenozide in litter and soil

Litter and soil substrates were prepared as above and subjected to 15 mm rain with two different droplet size spectra (i.e., rainfall with two intensities) after a rain-free period of 6 days post-treatment. The smaller rain droplets (diameter range 30 to 340  $\mu\text{m}$ ,  $D_{N,5}$  155 ( $\pm 25$ )  $\mu\text{m}$ ,  $D_{V,5}$  315 ( $\pm 25$ )  $\mu\text{m}$ ) were produced by using a Veejet® 8001 nozzle with an orifice of 0.65 mm and spray angle of 72°, at a pressure of 160 kPa and flow rate of 0.24 litre  $\text{min}^{-1}$ , and the duration of the rain was 15 h continuously at an intensity of 1 mm  $\text{h}^{-1}$ . The larger rain droplets were produced in the same manner as described in Section 2.2.1, and the 15 mm rain was applied over 3 h at an intensity of 5 mm  $\text{h}^{-1}$ . Samples of litter and soil were collected, processed and analyzed for tebufenozide residues.<sup>6</sup>

### 2.2.4 Effect of cumulative rainfall on lateral mobility of tebufenozide in litter and soil

An apparatus (Fig. 2) was constructed using a stainless steel circular pan (labelled CC-I, 30 cm ID, 6 cm high), and four concentric circular frames (CC-II, 24 cm ID, 7.5 cm high; CC-III, 18 cm ID, 12 cm high; CC-IV, 12 cm ID, 16 cm high; CC-V, 6.5 cm ID, 18 cm high) made of stainless steel gauze with mesh size 0.2 mm. The pan, CC-I, was filled with the litter or soil substrate (untreated) up to a height of 3 cm, and the four circular frames were filled with the same substrate (also untreated) to a height of 6, 9, 12 and 15 cm. Two such apparatus were used, and replicate samples were pooled.

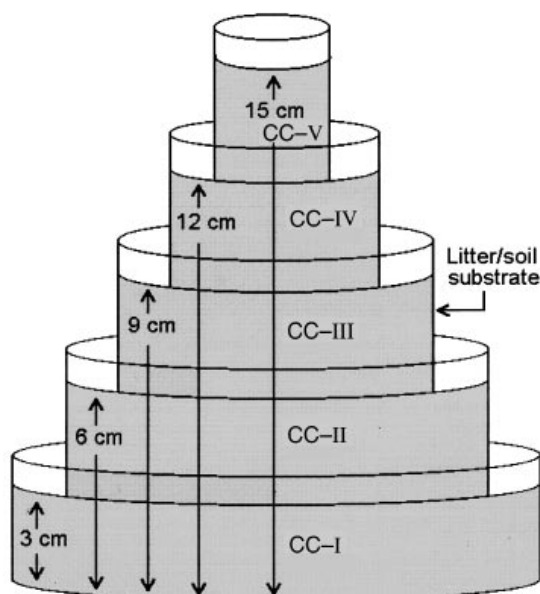


Fig. 2. Apparatus used to investigate the lateral mobility of tebufenozide due to simulated rainfall.

To determine lateral mobility of tebufenozide due to rainfall, the substrate (12 cm high) in the innermost circular frame, CC-V, was topped with a 3-cm layer of treated litter or soil containing an initial concentration of 10 mg AI  $\text{kg}^{-1}$ . Two replicate units of the apparatus were placed in the spray chamber in the same manner as the two Al pans used for the vertical mobility study (Fig. 1), and the substrates were subjected to continuous rainfall of 20 or 40 mm as described above. Immediately after the rain, both units were removed from the spray chamber and replicate cores (each 3 cm deep, 1 cm in diameter) were taken from the pan, CC-I and circular frames, CC-II to CC-V. The cores were sliced into three layers, each 1 cm deep, and replicate cores were pooled to provide three composite samples. The excess water was filtered using the same membrane filters as described above (PTFE™, 1- $\mu\text{m}$  pore, Gelman Sciences). The substrates (and the filtered water) were then analyzed for tebufenozide.<sup>6</sup>

### 2.2.5 Effect of light radiation and radiation intensity on disappearance of tebufenozide from litter and soil

Prior to the investigation, the intensity and emission spectra of the natural sunlight were measured in an area open to sunlight between 0900 and 1500 h every day for 20 consecutive days using a Spectroradiometer (Model LI-1800, LI-COR, Inc., Lincoln, Nebraska). The wavelength ranged from 320 to 1100 nm (Fig. 3), with an average intensity of 678  $\text{W m}^{-2}$  and a mean temperature of 16.5°C. Similar measurements made inside an environmental chamber (where the investigation was

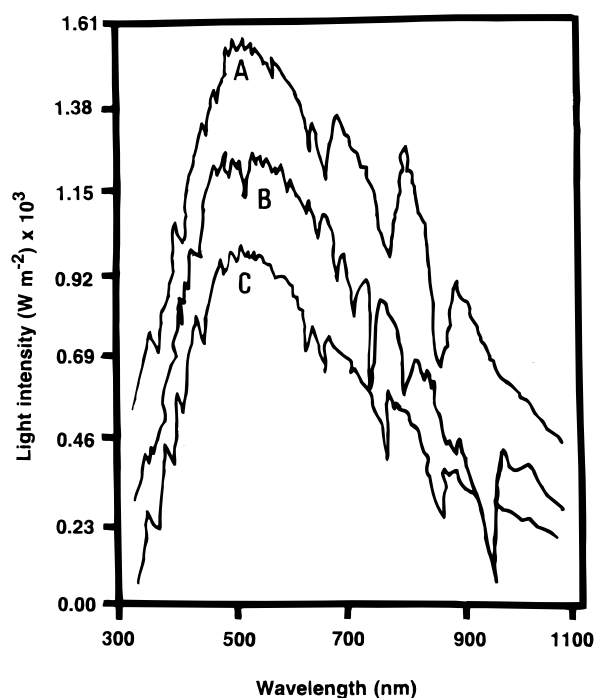


Fig. 3. Light intensity ( $\text{W m}^{-2}$ ) versus wavelength (nm) of radiation during photostability studies: (A) Intensity with four 100-W lamps; (B) Intensity of natural sunlight; (C) Intensity with two 100-W lamps.

carried out) at a height of 40 cm below two household lamps (100 W each) indicated the same wavelength range (Fig. 3), but with an intensity of  $495 \text{ W m}^{-2}$  (c. 73% of the value in the open sky), and a mean temperature of  $15^\circ\text{C}$ .

Litter and soil samples, treated with RH-5992 2F at  $10 \text{ mg AI kg}^{-1}$ , were packed (1 cm deep) in Al trays ( $24 \times 16 \text{ cm} \times 1 \text{ cm}$  deep), and stored in the environmental chamber in darkness until exposure to light radiation. Two lamps (100 W each) were placed at 40 cm above the substrates. The substrates were exposed to radiation (intensity,  $495 \text{ W m}^{-2}$ ) for 6 and 12 days after a radiation-free period of 5 days post-treatment, and samples (cores, 2 cm in diameter and 1 cm in depth) were analyzed for tebufenozide residues.<sup>6</sup>

Treated litter and soil samples were also packed in Al trays as above, and subjected to radiation at two different intensities (Fig. 3), after a radiation-free period of 8 days post-treatment. One set of samples was exposed to radiation intensity of  $495 \text{ W m}^{-2}$  as above. To obtain light radiation of higher intensity, two additional household lamps (100 W each) were used (i.e., four lamps in total). This increased the radiation intensity from  $495 \text{ W m}^{-2}$  to  $815 \text{ W m}^{-2}$  (c. 120% of the value in the open sky). The duration of exposure was maintained at 8 days for both intensities. After exposure, the samples (cores, 2 cm in diameter and 1 cm in depth) were analyzed for tebufenozide residues.<sup>6</sup>

#### 2.2.6 Effect of volatilization of tebufenozide from litter and soil on disappearance

Volatilization of tebufenozide from treated substrates was conducted in an apparatus shown in Fig. 4. A 200-g aliquot of each substrate, containing tebufenozide at a concentration of  $10 \text{ mg kg}^{-1}$ , was taken in a Buchner® flask (500 ml capacity). Air was allowed to pass through the substrate at a flow rate of  $0.75 \text{ litre min}^{-1}$ , and collected in two impingers,<sup>11,12</sup> containing acetonitrile as the trapping medium (Fig. 4). The apparatus (in duplicate) was placed in darkness in two environmental chambers maintained at 7 and  $30^\circ\text{C}$  respectively, and tebufenozide vapour was collected for 10 days contin-

uously. The impingers were removed every 24 h, the solvent in the two impingers was pooled into one sample, and a new set of impingers containing fresh acetonitrile was used. At the end of 10 days, the 10 acetonitrile samples were pooled into five composite samples, i.e., those collected on days 1 and 10 into sample 1; days 2 and 9 into sample II; days 3 and 8 into sample III; days 4 and 7 into sample IV and days 5 and 6 into sample V. These were concentrated by flash-evaporation at  $27^\circ\text{C}$  prior to analysis of tebufenozide.<sup>6</sup>

Another set of experiments was conducted to determine the increase in volatilization of tebufenozide when the time interval of passage of air through the substrates was increased. Air was allowed to pass through treated substrate samples (kept in darkness at a constant temperature of  $15^\circ\text{C}$ ) for eight and 20 days respectively, and tebufenozide vapour was collected. The acetonitrile samples were processed as above and tebufenozide residues were determined.<sup>6</sup>

### 3 RESULTS AND DISCUSSION

#### 3.1 Study I—Behaviour of tebufenozide in sandy litter and soil ecosystems under field conditions

##### 3.1.1 Residues of tebufenozide in sandy forest litter

The residues in sandy forest litter are given in Table 1. The data, measured on a dry weight basis, were adjusted to the constant, pre-spray moisture level of 27%. The initial average concentrations measured at 1 h post-spray (referred to as zero day in Table 1) increased in proportion to the dosage rates. Residues decreased gradually during the 408-day persistence study, although some fluctuations were observed in the concentration levels. This behaviour is difficult to explain, although rainfall occurring during the course of the study could have played some role. The long persistence observed in litter could be due to the strong binding between tebufenozide and particulates in the litter matrix. The high organic matter content (c. 90%), lipophilic nature, low acidity (pH 5.4) etc. (Table 1) could

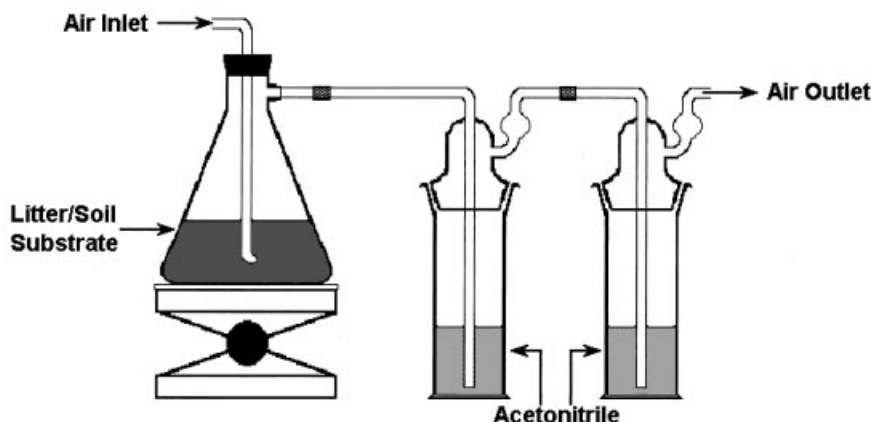


Fig. 4. Apparatus used in the volatilization study for trapping tebufenozide vapour.

**TABLE 1**  
Study I—Dissipation Kinetics<sup>a</sup> of Tebufenozide in Sandy Forest Litter<sup>b</sup>

Time after spray (days)	Rate applied (g AI ha <sup>-1</sup> )		
	35	70	140
	Concentration in litter (µg g <sup>-1</sup> wet weight) (±SD) <sup>c</sup>		
0	0.176 (±0.076)	0.338 (±0.162)	0.637 (±0.232)
4	0.133 (±0.048)	0.232 (±0.081)	0.531 (±0.179)
9	0.098 (±0.022)	0.226 (±0.072)	0.580 (±0.159)
16	0.091 (±0.031)	0.166 (±0.113)	0.535 (±0.138)
23	0.076 (±0.033)	0.189 (±0.162)	0.533 (±0.198)
31	0.099 (±0.046)	0.229 (±0.086)	0.497 (±0.266)
43	0.079 (±0.058)	0.172 (±0.066)	0.472 (±0.137)
52	0.084 (±0.031)	0.164 (±0.037)	0.456 (±0.219)
64	0.069 (±0.008)	0.134 (±0.032)	0.452 (±0.269)
85	0.062 (±0.005)	0.169 (±0.094)	0.396 (±0.112)
107	0.055 (±0.020)	0.144 (±0.071)	0.335 (±0.205)
135	0.082 (±0.026)	0.147 (±0.006)	0.313 (±0.057)
169	0.052 (±0.015)	0.131 (±0.050)	0.237 (±0.103)
357 <sup>d</sup>	T <sup>e</sup>	0.089 (±0.024)	0.291 (±0.112)
370 <sup>f</sup>	ND <sup>g</sup>	0.083 (±0.019)	0.242 (±0.141)
408 <sup>h</sup>	ND	0.072 (±0.021)	0.164 (±0.081)
Constant <i>B</i>	0.139	0.287	0.603
Coeff. detr. ( <i>R</i> <sup>2</sup> )	0.796	0.877	0.959
Rate constant <i>C</i>	0.001 11	0.002 31	0.002 63
DT <sub>50</sub> (days)	63.1	62.3	115

<sup>a</sup> Dissipation kinetics of residues followed the three equations,  $Y = B - Ct$ ,  $Y = 0.5B - C DT_{50}$  and  $DT_{50} = 0.5 B/C$ .

<sup>b</sup> Organic matter c. 90% of total mass; of remainder: sand 57%, silt 27%, clay 16%, pH 5.4.

<sup>c</sup> Calculated from six measurements of three replicate samples.

<sup>d</sup> Date of sampling = 25 May 1993.

<sup>e</sup> T = Traces, 0.02–0.049 µg g<sup>-1</sup>.

<sup>f</sup> Date of sampling = 7 June 1993.

<sup>g</sup> ND = Not detected (LOD, 0.02 µg g<sup>-1</sup>).

<sup>h</sup> Date of sampling = 15 July 1993.

have favoured adsorption and immobilization of tebufenozide to the litter matrix, thus acting as a micro-sink.<sup>13</sup>

The residual concentrations (individual values measured) obtained during the initial 85 days after spray were subjected to regression analysis, and were found to fit into eqns (1) to (3):

$$Y = B - Ct \quad (1)$$

$$Y = 0.5B = B - C DT_{50} \quad (2)$$

$$DT_{50} = 0.5B/C \quad (3)$$

where *Y* = residue concentration at time *t*, *B* = theoretical initial residue level, *C* = rate constant of the linear decline of residues, and DT<sub>50</sub>, the half-life (the time required for 50% of the initial residue to disappear) of persistence. Values of the constants, *B*, *C* and DT<sub>50</sub> are given in Table 1. The DT<sub>50</sub> values for the

plots treated at the three dosage rates were all high and ranged from 62.3 to 115 days. These high values suggest that tebufenozide could even overwinter after aerial application. However, it is worth noting that initial concentrations were high in the present study, probably because the material was applied close to the litter plots. High concentrations in forest substrates are known to take a much longer time to dissipate than low concentrations.<sup>13</sup> In aerial applications, however, the amount deposited on the forest floor is expected to be much lower than in the present study, and this could influence the DT<sub>50</sub> values. Further studies are required before any definite conclusions can be drawn on the undue persistence of tebufenozide in forest litter and soil samples.

### 3.1.2 Residues of tebufenozide in sandy forest soil

The concentrations of tebufenozide in sandy soil (cores of 0–2.5 cm depth), adjusted to the constant pre-spray

**TABLE 2**  
Study I—Dissipation Kinetics<sup>a</sup> of Tebufenozide in Sandy Forest Soil<sup>b</sup>

Time after spray (days)	Rate applied (g AI ha <sup>-1</sup> )		
	35	70	140
	Concentration in soil (0–2.5 cm layer) (µg g <sup>-1</sup> wet weight) (± SD) <sup>c</sup>		
0	0.154 (± 0.133)	0.338 (± 0.100)	0.696 (± 0.206)
4	0.116 (± 0.084)	0.232 (± 0.079)	0.461 (± 0.266)
9	0.089 (± 0.084)	0.226 (± 0.033)	0.685 (± 0.186)
16	0.088 (± 0.024)	0.166 (± 0.108)	0.527 (± 0.274)
23	0.094 (± 0.055)	0.189 (± 0.074)	0.357 (± 0.131)
31	0.084 (± 0.062)	0.229 (± 0.081)	0.262 (± 0.104)
43	0.066 (± 0.032)	0.172 (± 0.088)	0.305 (± 0.088)
52	0.069 (± 0.033)	0.164 (± 0.048)	0.414 (± 0.284)
64	0.056 (± 0.050)	0.134 (± 0.049)	0.384 (± 0.095)
85	0.057 (± 0.028)	0.169 (± 0.069)	0.180 (± 0.085)
107	T <sup>e</sup>	0.051 (± 0.034)	0.096 (± 0.039)
135	T	T	0.063 (± 0.023)
169	T	T	0.070 (± 0.037)
357 <sup>d</sup>	ND <sup>g</sup>	ND	0.078 (± 0.041)
370 <sup>f</sup>	ND	ND	T
408 <sup>h</sup>	ND	ND	T
Constant <i>B</i>	0.121	0.297	0.616
Coeff. detr. ( <i>R</i> <sup>2</sup> )	0.802	0.938	0.935
Rate constant <i>C</i>	0.000 98	0.002 40	0.005 88
DT <sub>50</sub> (days)	62.2	62.1	52.4

*a*, *c*, *d*, *e*, *f* and *g*: See footnotes to Table 1.

*b*: Organic matter 3.9% of total mass; of remainder: sand 51%, silt 42%, clay 7%, pH 5.9.

moisture level of 24.3%, are given in Table 2. The initial concentrations were similar to those measured in the equivalent litter plots (Table 1). The residues decreased gradually with time and lingered up to 408 days post-spray in samples collected from the plots sprayed at 140 g AI ha<sup>-1</sup>. The 169-day post-spray samples still contained about 10% of the initial deposits of tebufenozide, whereas samples from other plots, treated with the two lower dosages, contained only 'trace' (T) levels (0.02–0.049 µg g<sup>-1</sup>) (Table 2). Similar to the findings in litter samples, there were noticeable fluctuations in the soil concentrations over the post-spray period.

The residual concentrations (individual measurements) obtained during the initial 85-day post-spray period were found to fit into eqns (1) to (3), and DT<sub>50</sub> values were computed (Table 2). The values were about the same (62.2 and 62.1 days respectively) for the two dosage rates of 35 and 70 g AI ha<sup>-1</sup>, and were similar to those (63.1 and 62.3 days respectively) obtained for the litter (Table 1). At the highest dosage rate of 140 g AI ha<sup>-1</sup>, the soil samples showed a slightly lower DT<sub>50</sub> of 52.4 days, whereas the litter samples showed a higher value of 115 days. The reason for this discrepancy is unclear. The sandy litter was more lipophilic and contained more organic matter (about 90%) than the sandy soil (OM 3.9%), and therefore the DT<sub>50</sub> values might be expected to be higher in litter than in soil. In the present

study, however, the observed fluctuations in the DT<sub>50</sub> values could be due to irregular variations in rainfall, exposure to sunlight, volatilization and microbial content in the substrates, all of which would affect the persistence of tebufenozide under field conditions.

The high DT<sub>50</sub> values in soils, similar to those in litter, indicate that tebufenozide is likely to persist in the matrix for a long time. However, as mentioned above, further investigations are required using aerial applications before any definite conclusions can be drawn on the undue persistence of tebufenozide in forest soil matrices.

### 3.1.3 Vertical downward mobility in sandy forest soil

No tebufenozide was detected (LOD, 0.02 µg g<sup>-1</sup>) in soil samples collected from 2.5–5.0 cm depth during zero to 85 days post-spray (Table 3) irrespective of dosage rate. Samples from the plot sprayed at the highest dosage rate contained 0.05 µg g<sup>-1</sup> on the 107th and 408th day post-spray. All the other samples (2.5–5.0 cm cores) including the ones from 5.0–10 and 10–15 cm had no detectable amounts of tebufenozide except for the occasional incidence of 'trace' amounts (Table 3). The lack of downward mobility observed in the present study could be due to strong adsorption of tebufenozide onto the sandy soil.



**TABLE 3**  
Study I—Vertical Downward Mobility of Tebufenozide in Sandy Forest Soil

Time after spray (days)	Rates applied (g AI ha <sup>-1</sup> )		
	35	70	140
	Concentration ( $\mu\text{g g}^{-1}$ wet weight) in soil cores from 2.5 to 5.0 cm depth <sup>a</sup>		
0–85	ND <sup>b</sup>	ND	ND
107	T <sup>c</sup>	T	0.05
135	ND	T	T
169	ND	T	T
408	ND	T	0.05

a: No quantifiable residues in 5–10 and 10–15 cm cores.

b: ND = Not detectable (LOD, 0.02  $\mu\text{g g}^{-1}$ ).

c: 'T' = Trace (0.02–0.049  $\mu\text{g g}^{-1}$ ).

### 3.1.4 Droplet size spectra and deposit assessment

The average droplet parameters obtained on Kromekote cards for the litter and soil plots are given in Table 4. Neither  $D_{\max}$ ,  $D_{\min}$ ,  $D_{N.5}$ ,  $D_{V.5}$ , nor the droplet density showed marked differences among them, although the percentage distribution of droplet number or volume in different size categories indicated some differences, as shown in Fig. 5. However, such differences did not influence the  $D_{\max}$ ,  $D_{\min}$ ,  $D_{N.5}$ ,  $D_{V.5}$  or droplet density values markedly.

The average deposit of tebufenozide on glass plates ranged from about 88 to 95%, indicating a high level of deposition (Table 4). This is expected because spray was

applied close to the ground level (<0.5 m) within a polyethylene enclosure.

## 3.2 Study II—Behaviour of tebufenozide in sandy/clay litter and soil samples in laboratory microcosms

### 3.2.1 Effect of cumulative rainfall on vertical mobility of tebufenozide in litter and soil

After continuous and intermittent rainfall (Table 5) tebufenozide moved downward more in the sandy litter and soil than in the clay litter and soil. This behaviour was noted after both continuous and intermittent rainfall of 20 and 40 mm. However, continuous rainfall caused more downward movement than intermittent rainfall. In the 3 h rain-free interval between rainfalls, tebufenozide was probably adsorbed onto the moist substrates, thus decreasing downward mobility during the following rainfall. With the continuous rainfall, however, there seems not to have been enough time for tebufenozide to become adsorbed onto the substrates.

As expected, the 40 mm rain caused more downward mobility than did the 20 mm. This was noted with both continuous and intermittent rainfall. No residues were found in the bottom layer of either sandy or clay substrates after 20 mm rain, compared to those found (0.17 to 0.45  $\mu\text{g g}^{-1}$ ) after 40 mm rain (Table 5). With the intermittent rain, however, the middle 2-cm layer showed no measurable residues after 20 mm rain, and residues were present only after 40 mm rain. The bottom layer did not show any residues after 20 or 40 mm rainfall.

The amount of tebufenozide found in the filtered water was probably due to its solubility [c. 0.83 mg litre<sup>-1</sup> (0.83 mg kg<sup>-1</sup>, density of water  $\approx 1.00$ )]. However, it is worth noting that the residues found in water were lower than the solubility limit, and ranged from 0.101 to 0.437 mg kg<sup>-1</sup> depending on the substrate layer from which the water was filtered. It

**TABLE 4**

Study I—Pooled Mean Values<sup>a</sup> of Droplet Parameters and Spray Mass Deposits Obtained on Artificial Samplers in Litter and Soil Plots After Application of Tebufenozide at Three Dosage Rates With a Constant Volume Rate of 2 litre ha<sup>-1</sup>

Parameters	Rates applied (g AI ha <sup>-1</sup> )		
	35	70	140
<i>Droplet parameters on Kromekote cards:</i>			
$D_{\max}$ ( $\mu\text{m}$ )	135	143	140
$D_{\min}$ ( $\mu\text{m}$ )	16	17	11
$D_{N.5}$ ( $\mu\text{m}$ )	79	78	76
$D_{V.5}$ ( $\mu\text{m}$ )	84	97	93
Droplets cm <sup>-2</sup>	57	45	53
<i>Spray deposits on glass plates:</i>			
Deposit of tebufenozide (g AI ha <sup>-1</sup> )	30.75	65.65	132.9
Deposition (%)	87.86	93.79	94.93

a: Pooled mean values refer to the means of data obtained in litter and soil plots at each of the three dosages of 35, 70 and 140 g AI ha<sup>-1</sup> respectively.

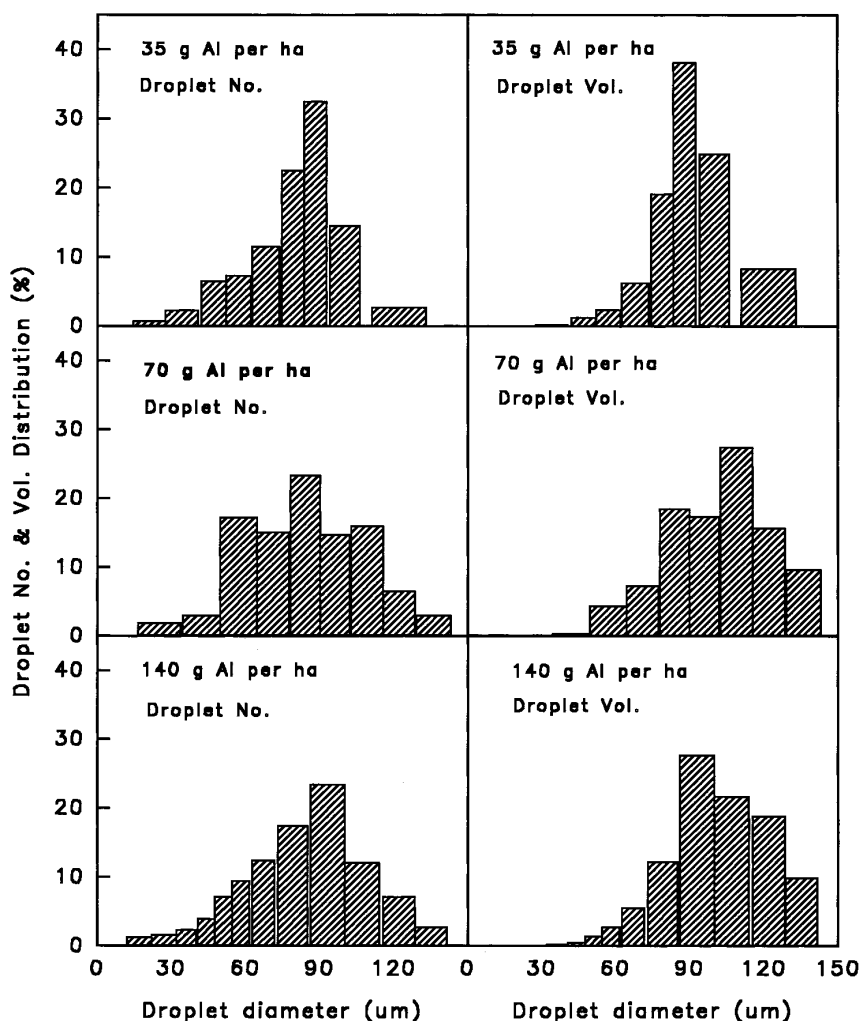


Fig. 5. Droplet number and volume distribution percentages according to size category for tebufenozide spray mixtures applied to litter and soil plots at 35, 70 and 140 g AI in 2 litre ha<sup>-1</sup>.

appears that during the time of rainfall, tebufenozide never reached its maximum solubility limit in water.

### 3.2.2 Effect of rain-free period on vertical mobility of tebufenozide in litter and soil

The data in Table 6 indicate that downward mobility occurred more after a three-day rain-free period than after a 12-day period. For example, more residues were present in the middle layer when a 30-mm rainfall occurred at three days post-treatment than at 12 days. In fact, residues in the bottom 2-cm layer were still measurable after a three-day rain-free period, but were below the detection limit when the same amount of rain occurred at 12 days post-treatment. This was noted both with sandy and clay substrates. It appears that the longer the rain-free period, the greater the time available for adsorption of the chemical onto the active sites of the substrates.

Similar to the finding after the 20 and 40 mm cumulative rainfall, more downward mobility occurred after 30 mm rainfall with the sandy substrates than with the

clay substrates. This is probably due to the greater adsorptive capacity of the clay substrates than that of the sandy substrates, in agreement with the findings reported previously.<sup>14,15</sup>

### 3.2.3 Effect of rainfall intensity on vertical mobility of tebufenozide in litter and soil

After a cumulative rain of 15 mm, downward mobility occurred more with the rainfall of higher intensity (i.e. larger droplet size with 5 mm h<sup>-1</sup>) than with the same amount of rainfall of lower intensity (i.e. smaller droplet size with 1 mm h<sup>-1</sup>) (Table 6). This behaviour was noted with both the sandy and clay substrates. For example, no residues were found in the middle 2-cm layer of the substrates after the 15-mm cumulative rainfall with  $D_{V,5}$  of 315 ( $\pm 25$ )  $\mu\text{m}$  (intensity of 1 mm h<sup>-1</sup>), whereas residues were measurable after the same amount of rain with a  $D_{V,5}$  of 780 ( $\pm 95$ )  $\mu\text{m}$  (intensity of 5 mm h<sup>-1</sup>) (Table 6). The larger the rain droplet size, the greater the impact velocity of the droplets, resulting

TABLE 5

Study II—Effect of Cumulative Rainfall (Continuous and Intermittent) on Vertical Downward Mobility of Tebufenozide from Treated Sandy/Clay Litter and Soil Layers into Bottom Untreated Layers

Parameter	Sandy litter <sup>a,b</sup> (Filtered water) <sup>c</sup>	Sandy soil <sup>a,b</sup> (Filtered water) <sup>c</sup>	Clay litter <sup>a,b</sup> (Filtered water) <sup>c</sup>	Clay soil <sup>a,b</sup> (Filtered water) <sup>c</sup>
Pre-rain residues (mg kg <sup>-1</sup> )				
Top 2 cm	10.0 (NA) <sup>d</sup>	10.0 (NA)	10.0 (NA)	10.0 (NA)
Middle 2 cm	ND <sup>e</sup>	ND	ND	ND
Bottom 2 cm	ND	ND	ND	ND
Post-rain residues (mg kg <sup>-1</sup> ) after 20 mm continuous rainfall at a rain-free period of two days after treatment				
Top 2 cm	8.23 (0.421)	7.14 (0.430)	9.33 (0.411)	8.56 (0.395)
Middle 2 cm	0.85 (0.377)	1.05 (0.369)	0.72 (0.382)	0.97 (0.375)
Bottom 2 cm	ND (NS) <sup>f</sup>	ND (NS)	ND (NS)	ND (NS)
Post-rain residues (mg kg <sup>-1</sup> ) after 40 mm continuous rainfall at a rain-free period of two days after treatment				
Top 2 cm	6.54 (0.405)	5.33 (0.403)	7.82 (0.391)	6.65 (0.433)
Middle 2 cm	1.35 (0.365)	1.45 (0.347)	0.93 (0.387)	1.19 (0.357)
Bottom 2 cm	0.36 (0.101)	0.45 (0.123)	0.17 (0.134)	0.43 (0.127)
Post-rain residues (mg kg <sup>-1</sup> ) after 20 mm intermittent <sup>g</sup> rainfall at a rain-free period of two days after treatment				
Top 2 cm	9.13 (0.404)	8.44 (0.412)	10.0 (0.437)	9.77 (0.427)
Middle 2 cm	ND (NS)	ND (NS)	ND (NS)	ND (NS)
Bottom 2 cm	ND (NS)	ND (NS)	ND (NS)	ND (NS)
Post-rain residues (mg kg <sup>-1</sup> ) after 40 mm intermittent <sup>g</sup> rainfall at a rain-free period of two days treatment				
Top 2 cm	7.74 (0.429)	6.87 (0.403)	8.19 (0.411)	7.47 (0.391)
Middle 2 cm	0.95 (0.399)	1.23 (0.338)	0.75 (0.326)	0.99 (0.343)
Bottom 2 cm	ND (NS)	ND (NS)	ND (NS)	ND (NS)

a: For organic matter, sand, silt, clay and pH values of sandy litter and soil, see Tables 1 and 2.

The corresponding values for the clay litter: OM c. 80%, sand 21%, silt 35%, clay 44%, pH 5.2; and for the clay soil: OM c. 3%, sand 18%, silt 40%, clay 42%, pH 5.3.

b: Residue data refer to the mean of three replicate samples.

c: Values in parenthesis refer to the amount found in the filtered water.

d: NA = not applicable.

e: ND = not detectable (LOD, 0.020 mg kg<sup>-1</sup>).

f: NS = samples were not soaked in rain water, because the rain water did not reach the bottom layer.

g: The 20 and 40 mm rainfall were applied in four applications, each of 5 or 10 mm respectively.

in a forceful movement of the tebufenozide from the top to the middle layer.

### 3.2.4 Effect of cumulative rainfall on lateral mobility of tebufenozide in litter and soil

The data in Table 7 indicate that after the 20 mm rainfall, little downward movement of tebufenozide

occurred in the innermost frame, CC-V, from the top 1-cm layer to the middle and bottom layers (1 cm each) because the residue concentration measured in the middle 1-cm layer (9.27 to 9.95 mg kg<sup>-1</sup>) was close to the initial level treated (10.0 mg kg<sup>-1</sup>). A similar finding was also noted after the 40 mm rainfall. Nevertheless, appreciable amounts of tebufenozide were found in the top and middle 1-cm layers of the untreated substrates

TABLE 6

Study II—Effect of Rain-Free Period and Rainfall Intensity on Vertical Downward Mobility of Tebufenozide from Treated Sandy/Clay Litter and Soil Layers into Bottom Untreated Layers

Parameter	Sandy litter <sup>a,b</sup> (Filtered water) <sup>c</sup>	Sandy soil <sup>a,b</sup> (Filtered water) <sup>c</sup>	Clay litter <sup>a,b</sup> (Filtered water) <sup>c</sup>	Clay soil <sup>a,b</sup> (Filtered water) <sup>c</sup>
Pre-rain residues (mg kg <sup>-1</sup> )				
Top 2 cm	10.0 (NA) <sup>d</sup>	10.0 (NA)	10.0 (NA)	10.0 (NA)
Middle 2 cm	ND <sup>e</sup>	ND	ND	ND
Bottom 2 cm	ND	ND	ND	ND
Post-rain residues (mg kg <sup>-1</sup> ) after 30 mm rainfall at a rain-free period of three days after treatment				
Top 2 cm	7.39 (0.391)	6.22 (0.402)	8.13 (0.398)	7.67 (0.435)
Middle 2 cm	1.22 (0.394)	1.01 (0.366)	0.71 (0.377)	0.82 (0.345)
Bottom 2 cm	0.16 (0.090)	0.25 (0.109)	0.09 (0.123)	0.23 (0.118)
Post-rain residues (mg kg <sup>-1</sup> ) after 30 mm rainfall at a rain-free period of 12 days after treatment				
Top 2 cm	9.44 (0.451)	8.33 (0.437)	9.92 (0.403)	9.01 (0.383)
Middle 2 cm	0.38 (0.335)	0.69 (0.344)	0.27 (0.307)	0.36 (0.327)
Bottom 2 cm	ND (0.097)	ND (0.131)	ND (0.076)	ND (0.094)
Post-rain residues (mg kg <sup>-1</sup> ) after 15 mm rainfall with intensity of 1 mm h <sup>-1</sup> at a rain-free period of six days after treatment				
Top 2 cm	9.03 (0.424)	8.00 (0.419)	9.83 (0.405)	8.77 (0.444)
Middle 2 cm	ND (NS) <sup>f</sup>	ND (NS)	ND (NS)	ND (NS)
Bottom 2 cm	ND (NS)	ND (NS)	ND (NS)	ND (NS)
Post-rain residues (mg kg <sup>-1</sup> ) after 15 mm rainfall with intensity of 5 mm h <sup>-1</sup> at a rain-free period of six days after treatment				
Top 2 cm	7.84 (0.459)	6.43 (0.409)	8.99 (0.424)	7.77 (0.372)
Middle 2 cm	1.25 (0.353)	1.33 (0.341)	0.86 (0.317)	1.03 (0.322)
Bottom 2 cm	ND (NS)	ND (NS)	ND (NS)	ND (NS)

a: For organic matter, sand, silt, clay and pH values of sandy litter and soil, see Tables 1 and 2.

For those of clay litter and soil see Table 5.

b, c, d, e and f: See footnotes to Table 5.

of CC-IV and CC-III. This must have been carried by water passing laterally through the gauze surrounding the treated layer in CC-V and falling onto the surface of CC-IV and then CC-III. After 40 mm rainfall, this was sufficient to give measurable residues even in the bottom layer in CC-IV. Thus appreciable lateral movement of tebufenozide can occur under the influence of rainwater moving away from the treated area, for example, down a slope.

The residues found in the filtered water after 20 and 40 mm rainfall, were similar to those observed in the vertical movement investigations, and ranged from

‘traces’ (0.02 to 0.049 mg kg<sup>-1</sup>) to 0.457 mg kg<sup>-1</sup>. These values, once again, are below the solubility limit of tebufenozide in water.

### 3.2.5 Effect of light radiation and radiation intensity on disappearance of tebufenozide from litter and soil

The data in Table 8 indicate that loss of tebufenozide occurred more from sandy substrates than from clay substrates. This behaviour was noted regardless of the intensity of radiation and duration of exposure. Once again, this is probably due to the greater adsorptive capacity of the clay substrates than that of the sandy

TABLE 7

Study II—Effect of Cumulative Rainfall on Vertical and Lateral Mobility of Tebufenozide from Treated Sandy/Clay Litter and Soil Layers into Untreated Layers

Parameter	Sandy litter <sup>a,b</sup> (Filtered water) <sup>c</sup>	Sandy soil <sup>a,b</sup> (Filtered water) <sup>c</sup>	Clay litter <sup>a,b</sup> (Filtered water) <sup>c</sup>	Clay soil <sup>a,b</sup> (Filtered water) <sup>c</sup>
Post-rain residues (mg kg <sup>-1</sup> ) in CC-V after 20 mm rainfall at a rain-free period of four days after treatment				
Top 1 cm	8.79 (0.454)	7.89 (0.423)	9.05 (0.413)	8.35 (0.457)
Middle 1 cm	9.85 (0.232)	9.43 (0.156)	9.95 (0.176)	9.27 (0.275)
Bottom 1 cm	10.0 (NS) <sup>f</sup>	9.95 (NS)	10.2 (NS)	9.99 (NS)
Post-rain residues (mg kg <sup>-1</sup> ) in CC-IV after 20 mm rainfall at a rain-free period of four days after treatment				
Top 1 cm	5.03 (0.394)	4.50 (0.409)	4.97 (0.385)	4.03 (0.414)
Middle 1 cm	0.15 (T) <sup>h</sup>	0.23 (T)	0.13 (T)	0.22 (T)
Bottom 1 cm	ND <sup>e</sup> (NS)	ND (NS)	ND (NS)	ND (NS)
Post-rain residues (mg kg <sup>-1</sup> ) in CC-III after 20 mm rainfall at a rain-free period of four days after treatment				
Top 1 cm	2.85 (0.359)	3.75 (0.380)	2.99 (0.383)	3.67 (0.395)
Middle 1 cm	T (T)	T (T)	T (T)	T (T)
Bottom 1 cm	ND (NS)	ND (NS)	ND (NS)	ND (NS)
Post-rain residues (mg kg <sup>-1</sup> ) in CC-V after 40 mm rainfall at a rain-free period of four days after treatment				
Top 1 cm	7.24 (0.414)	6.63 (0.408)	8.12 (0.435)	7.17 (0.422)
Middle 1 cm	8.95 (0.331)	7.97 (0.304)	9.85 (0.276)	8.77 (0.173)
Bottom 1 cm	10.38 (NS)	9.91 (NS)	10.28 (NS)	9.09 (NS)
Post-rain residues (mg kg <sup>-1</sup> ) in CC-IV after 40 mm rainfall at a rain-free period of four days after treatment				
Top 1 cm	6.14 (0.404)	5.03 (0.319)	5.55 (0.365)	4.99 (0.324)
Middle 1 cm	0.95 (0.222)	1.23 (0.195)	0.75 (0.176)	0.96 (0.134)
Bottom 1 cm	0.25 (0.077)	0.37 (0.089)	0.12 (0.088)	0.23 (0.096)
Post-rain residues (mg kg <sup>-1</sup> ) in CC-III after 40 mm rainfall at a rain-free period of four days after treatment				
Top 1 cm	3.95 (0.427)	4.43 (0.309)	2.92 (0.314)	3.70 (0.302)
Middle 1 cm	1.25 (0.358)	1.33 (0.371)	0.86 (0.333)	1.03 (0.379)
Bottom 1 cm	T (T)	T (T)	T (T)	T (T)

a: For organic matter, sand, silt, clay and pH values of sandy litter and soil, see Tables 1 and 2. For those of clay litter and soil see Table 5.

b, c, e, and f: See footnotes to Table 5.

h: Traces = values between 0.02 and 0.049 mg kg<sup>-1</sup>.

substrates, in agreement with the findings reported previously.<sup>14,15</sup>

The disappearance of tebufenozide showed a direct relationship with the duration of exposure, since a

greater amount disappeared after exposure to light of intensity 495 W m<sup>-2</sup> for 12 days than for six days. Similarly, higher amounts of tebufenozide were lost from the substrates exposed to light of high intensity

**TABLE 8**  
Study II—Effect of Light Radiation on Disappearance of Tebufenozide from Treated Sandy/Clay Litter and Soil Layers

<i>Sandy litter<sup>a,b</sup></i>	<i>Sandy soil<sup>a,b</sup></i>	<i>Clay litter<sup>a,b</sup></i>	<i>Clay soil<sup>a,b</sup></i>
Pre-radiation residues (mg kg <sup>-1</sup> )			
10.0	10.0	10.0	10.0
Post-radiation residues (mg kg <sup>-1</sup> ) after exposure to 495 W m <sup>-2</sup> for six days at a radiation-free period of five days after treatment			
8.17	7.56	8.85	7.89
Post-radiation residues (mg kg <sup>-1</sup> ) after exposure to 495 W m <sup>-2</sup> for 12 days at a radiation-free period of five days after treatment			
7.40	6.66	7.83	7.09
Post-radiation residues (mg kg <sup>-1</sup> ) after exposure to 495 W m <sup>-2</sup> for eight days at a radiation-free period of eight days after treatment			
6.74	5.69	7.28	6.01
Post-radiation residues (mg kg <sup>-1</sup> ) after exposure to 815 W m <sup>-2</sup> for eight days at a radiation-free period of eight days after treatment			
6.04	5.06	6.51	5.44

*a*: For organic matter, sand, silt, clay and pH values of sandy litter and soil, see Tables 1 and 2. For those of clay litter and soil see Table 5.

*b*: See footnote to Table 5.

(815 W m<sup>-2</sup>) than to light of low intensity (495 W m<sup>-2</sup>), after the same duration (eight days) of exposure.

### 3.2.6 Effect of volatilization of tebufenozide from litter and soil on disappearance

The data in Table 9 show that volatilization of tebufenozide occurred more at 30°C than at 7°C, indicating that the process is related to temperature. However, when the temperature was maintained at 15°C, a longer duration of passage of air caused a greater loss of tebu-

fenozide. This behaviour was noted with both types of substrate, i.e., sandy and clay types. Nonetheless, tebufenozide volatilized more from substrates of the sandy type than those of the clay type, again probably because of the greater adsorption occurring onto the clay substrates than onto the sandy substrates.<sup>14,15</sup>

## 4 CONCLUSIONS

The present investigation indicated that, under field conditions, tebufenozide persisted in sandy litter and

**TABLE 9**  
Study II—Effect of Volatilization on Disappearance of Tebufenozide from Treated Sandy/Clay Litter and Soil Samples

<i>Sandy litter<sup>a,b</sup></i>	<i>Sandy soil<sup>a,b</sup></i>	<i>Clay litter<sup>a,b</sup></i>	<i>Clay soil<sup>a,b</sup></i>
Pre-volatilization residues (mg kg <sup>-1</sup> )			
10.0	10.0	10.0	10.0
Post-volatilization residues (mg kg <sup>-1</sup> ) in samples kept in darkness at 7°C for 10 days			
9.49	9.01	9.85	9.46
Post-volatilization residues (mg kg <sup>-1</sup> ) in samples kept in darkness at 30°C for 10 days			
8.89	8.29	9.02	8.45
Post-volatilization residues (mg kg <sup>-1</sup> ) in samples kept in darkness at 15°C for eight days			
8.56	7.66	9.08	7.92
Post-volatilization residues (mg kg <sup>-1</sup> ) in samples kept in darkness at 15°C for 20 days			
7.33	6.77	8.29	7.06

*a*: For organic matter, sand, silt, clay and pH values of sandy litter and soil, see Tables 1 and 2. For those of clay litter and soil see Table 5.

*b*: Residue data refer to the mean of five replicate samples.

soil for a considerable length of time. The  $DT_{50}$  values were *c.* 60 days for both the substrates treated with the dosage rates of 35 and 70 g AI ha<sup>-1</sup>. At the highest dosage rate (140 g AI ha<sup>-1</sup>), however, the  $DT_{50}$  was higher for the litter (115 days) than for the soil (*c.* 52 days), indicating irregular variations in persistence. The sandy litter was more lipophilic and contained more organic matter (*c.* 90%) than the sandy soil (OM *c.* 4%), and therefore tebufenozide is likely to persist for a longer period in the litter than in the soil. Nonetheless, the variations observed in the present study could be due to fluctuations in rainfall, exposure to sunlight, volatilization, and microbial content in the two substrates during the post-spray period. The  $DT_{50}$  values reported in the literature for another field study<sup>4</sup> were 52.9, 51.7 and 60.3 days in clay litter treated with the dosage rates of 35, 70 and 140 g AI ha<sup>-1</sup> respectively. The corresponding values for the clay soil were lower, 34.0, 32.9 and 32.1 days respectively. The clay litter and soil had an organic content of *c.* 80 and 3% respectively, and therefore the observed longer persistence of tebufenozide in clay litter than in clay soil was probably due to the higher organic matter content. Nevertheless, the  $DT_{50}$  values obtained in the present study for the sandy substrates are in disagreement with the values reported<sup>4</sup> for the clay substrates. It appears that several other factors (see above) could have played a role in affecting the persistence. Vertical mobility of tebufenozide from the top 2.5-cm layer of the sandy soil to the bottom 2.5–5.0-cm layers was minimal, and only traces were found during the post-spray period of 107 to 408 days. This observation is in agreement with that reported previously<sup>4</sup> for the clay soil, since tebufenozide residues were below the quantifiable limit (0.05 µg g<sup>-1</sup>) in layers 2.5–7.5 cm.

The laboratory microcosm studies indicated that measurable concentrations of tebufenozide moved from the top 2-cm layer to untreated layers beneath as a result of simulated rainfall. Downward mobility increased when the cumulative rainfall increased from 20 to 40 mm, and also when the rain was continuous rather than intermittent. Movement of tebufenozide was also influenced by the rain-free period (*i.e.*, the time interval between tebufenozide treatment and occurrence of rainfall), because the shorter the rain-free period the greater the downward movement. Rainfall intensity (*i.e.*, rain droplet size spectra) also contributed to downward mobility, because more residues were found in the untreated bottom layers when the rain droplet sizes were large ( $D_{V,5}$ , 780 µm) than when they were small ( $D_{V,5}$ , 315 µm). When rainwater could move laterally along the surface, as would occur in practice on a sloping site, this caused more lateral movement of tebufenozide (*i.e.* along the surface) than vertical movement into the soil.

The findings of the present laboratory study on vertical mobility in sandy soil are in disagreement with the

observation made in the field study, since little tebufenozide moved from the top 2.5-cm soil to bottom layers (2.5–5.0, 5–10 and 10–15 cm). Similar disagreement could also be found with findings of the field study reported in clay soil.<sup>4</sup> The reason could be that tebufenozide was probably more strongly adsorbed to both sandy and clay soils under field conditions than under laboratory conditions, thus resisting downward movement. Or, rainfall probably did not occur in the field study for a long time after spraying tebufenozide, thus providing a longer time interval for adsorption. It is also likely that the rainfall intensity was lower in the field study than in the laboratory study.

The laboratory investigation on photolysis indicated that the disappearance of tebufenozide showed a direct relationship with duration of exposure and also with radiation intensity. This finding is in agreement with that reported in the literature,<sup>16</sup> since tebufenozide was found to be susceptible to degradation after exposure to sunlight and ultraviolet radiation. The study on volatilization of tebufenozide under controlled conditions indicated that the amount lost depended on the ambient temperature and duration of air passing through the substrates. Nonetheless, the amount lost by volatilization was much lower than the amount lost by rainfall and exposure to radiation, thus indicating the greater importance of rainfall and sunlight radiation on field persistence, than of volatilization.

In all of the laboratory microcosm studies, more tebufenozide was lost from the sandy litter and soil than from clay litter and soil. This behaviour was probably due to the greater adsorptive capacity of the clay substrates than that of the sandy substrates, in agreement with the findings reported previously.<sup>14,15</sup>

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